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Change: Appeal number

Date of entry 05-10-2005

Publication numbers, publication type and publication dates


EP0481790 A2 22-04-1992 [1992/17]

EP0481790 A3 14-04-1993 [1993/15]

EP0481790 B1 24-02-1999 [1999/08]

Date of grant

24-02-1999 [1999/08]

Application numbers and filing dateEP19910309595 (91309595.6) 

Date of filing

17-10-1991 [1992/17]

Date of publication of search reportDate of publication of
search report

14-04-1993 [1993/15]

Priority number, priority date

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Classification (IPC) and bulletin numberC12N15/13, C12P21/08, C12N5/10, A61K39/395, // (C12N5/10, C12R1:91),
(C12P21/08, C12R1:91) [1992/17]**Designated states**

AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE [1992/17]

English title

Antibody production [1992/17]

French title

Production d'anticorps [1992/17]

German title

Antikörperherstellung [1992/17]

Designated states, applicant name, address

FOR ALL DESIGNATED STATES

THE WELLCOME FOUNDATION LIMITED

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Inventor name, address01 / Page, Martin John / Glaxo Wellcome plc, Gunnels Wood Road /
Stevenage, Hertfordshire SG1 2 NY / GB02 / Crowe, James Scott / Glaxo Wellcome plc, Gunnels Wood Road /
Stevenage, Hertfordshire SG1 2NY / GB [1997/19]**Representative name, address**

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Filing language

EN

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Publication language

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EN [1999/08]

A2

EN [1992/17]

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MUNICH/(+49-89) 23994465

Examination procedure

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29-10-1991 [1992/17]

request for accelerated

examination filed

09-02-1996

- accepted (yes/no) / date yes / 18-03-1996

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limit/reply 13-11-1997/M04/14-01-1998
24-07-1996/M04/22-11-1996
18-03-1996/M04/26-06-1996

communication R.51(4)
dispatched 14-04-1998
- approval (yes or no) no
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payment of fee for
grant/fee for printing 16-10-1998/16-10-1998

Divisional Application(s)

Divisional
application/publication
number(s) EP20020076251/EP1247865
EP20040077314/EP1484402
EP19970201842/EP0822255

Miscellaneous

(deleted) [1999/08]

Opposition procedure

01(09-06-1999)(06-11-2003)-WITHDRAWN
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02(26-10-1999)(18-11-1999)-ADMISSIBLE
Boehringer Ingelheim GmbH Patente
Binger Str. 173
55216 Ingelheim am Rhein/DE
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Kläs, Heinz-Gerd
Boehringer Ingelheim GmbH, Abteilung Patente
55216 Ingelheim/Rhein/DE

03(12-11-1999)(29-11-1999)-ADMISSIBLE
Cambridge Antibody Technology Limited
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Walton, Seán Malcolm
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04(23-11-1999)(14-01-2000)-ADMISSIBLE
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06(24-11-1999)(06-12-1999)-ADMISSIBLE
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07(24-11-1999)(30-04-2001)-WITHDRAWN
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08(24-11-1999)(07-11-2003)-WITHDRAWN
 Chugai Pharmaceutical Co., Ltd.
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observation of the proprietor to opposition
 date dispatch/time-
 limit/reply 21-02-2000/M09/01-12-2000
 revocation of the patent A.102(1)
 date dispatch/legal effect
 date 18-10-2002/18-01-2005 [2005/16]
 Date of oral
 proceedings/minutes 15-01-2002/27-05-2002

Appeal procedure

Appeal following opposition
 appeal received 18-12-2002
 statement of grounds filed 28-02-2003
 file number T0278/03-3308
 result of the appeal procedure
 APPEAL OF
 PROPRIETOR REJECTED 18-01-2005
 Date of oral
 proceedings/minutes 18-01-2005/21-01-2005

Renewal fees

Renewal fee A.86 (patent
 year / paid) 03/05-08-1993
 04/03-08-1994
 05/04-08-1995
 06/09-10-1996
 07/10-10-1997
 08/12-10-1998

Documents cited in the European Search

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 [Y] JOURNAL OF BIOLOGICAL CHEMISTRY. (MICROFILMS) vol. 263, no.
 13, 5 May 1988, BALTIMORE, MD US pages 6344 - 6355 Kaetzel,
 D.M.; Nilson, J.H.; 'Methotrexate-induced amplification of
 the bovine lutropin gens in chine hamster ovary cells.'
 [Y] METHODS IN ENZYMOLOGY vol. 185, 1989, pages 537 - 566
 KAUFMAN, R.; 'Gene Expression technology; Selection and
 coamplification of heterologous genes in mammalian cells'
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 BALTIMORE US pages 3011 - 3016 Wood, Clive R.; Dorner,

Andrew J.; Morris, George E.; Alderman, Edward M.; Wilson, Douglas; O'Hara, Richard M., Jr.; Kaufman, Rand 'High level synthesis of immunoglobulins in Chinese hamster ovary cells.'

Documents cited in the Examination Procedure

WO8901783 A2

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[End of Data]

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15-02-2006 09:57:51

ANTRAG AUF ERTEILUNG EINES EUROPÄISCHEN PATENTS / REQUEST FOR GRANT OF A EUROPEAN PATENT / REQUETE EN DELIVRANCE D'UN BREVET EUROPEEN

Bestätigung einer bereits durch Telekopie (Telefax) eingereichten Anmeldung /
Confirmation of an application already filed by facsimile /
Confirmation d'une demande déjà déposée par télécopie


Wenn ja, Datum der Übermittlung der Telekopie und Name der Einreichungsbehörde /
If yes, facsimile date and name of the authority with which the documents were filed /
Si oui, date d'envoi de la télécopie et nom de l'autorité de dépôt

Reçu:	Ja / Yes / Oui
29 -10- 1991	
10	ZEICHN.

Behörde /
Authority / Autorité

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration			
Anmeldenummer / Application No. / N° de la demande	MKEY	1	91309595.6
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	2	17 OCT 1991
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	3	29.10.91 291091
Anmeldetag / Date of filing / Date de dépôt		4	
Tabulatoren-Positionen / Tabulation marks / Arrêts de tabulation			
Es wird die Erteilung eines europäischen Patents und gemäß Artikel 94 die Prüfung der Anmeldung beantragt / Grant of a European patent, and examination of the application under Article 94, are hereby requested / Il est demandé la délivrance d'un brevet européen et, conformément à l'article 94, l'examen de la demande	EXAM 4	5	<input checked="" type="checkbox"/>
Zeichen des Anmelders oder Vertreters (max. 15 Positionen) / Applicant's or representative's reference (maximum 15 spaces) / Référence du demandeur ou du mandataire (max. 15 caractères ou espaces)	AREF	6	PA1195/MJS
ANMELDER / APPLICANT / DEMANDEUR Name / Nom		7	THE WELLCOME FOUNDATION LIMITED
Anschrift / Address / Adresse		8	UNICORN HOUSE, 160 EUSTON ROAD LONDON NW1 2BP ENGLAND
APPR 01 # 201157091			
# DEST #			
Zustellanschrift / Address for correspondence / Adresse pour la correspondance		9	STOTT, MICHAEL JOHN THE WELLCOME RESEARCH LABORATORIES GROUP PATENTS & AGREEMENTS LANGLEY COURT, BECKENHAM KENT BR3 3BS, ENGLAND
PADR		10	ENGLAND
Staat des Wohnsitzes oder Sitzes / State of residence or of principal place of business / Etat du domicile ou du siège		11	BRITISH
Staatsangehörigkeit / Nationality / Nationalité		12	081 658 2211
Telefon / Telephone / Téléphone		13	23937 081 658 6284
Telex / Télex	Telefax / Fax / Télécopie	14	
Weitere(r) Anmelder auf Zusatzblatt / Additional applicant(s) on additional sheet / Autre(s) demandeur(s) sur feuille additionnelle		15	STOTT, MICHAEL JOHN
VERTRETER / REPRESENTATIVE / MANDATAIRE: Name / Nome		16	THE WELLCOME RESEARCH LABORATORIES GROUP PATENTS & AGREEMENTS LANGLEY COURT, BECKENHAM KENT BR3 3BS, ENGLAND
(Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird / Name only one representative, who is to be listed in the Register of European Patents and to whom notification is to be made / N'indiquer qu'un seul mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)		17	081 658 2211
FREP 01 0413740 # 1 #		18	23937 081 650 6284
Geschäftsanschrift / Address of place of business / Adresse professionnelle		19	
Telefon / Telephone / Téléphone			
Telex / Télex	Telefax / Fax / Télécopie		
Weitere(r) Vertreter auf Zusatzblatt / Additional representative(s) on additional sheet / Autre(s) mandataire(s) sur feuille additionnelle			

27.11.95 CH

Patentansprüche / Claims / Revendications	CLMS	37	23 ✓	Zahl der Patentansprüche / Number of claims / Nombre de revendications
Weiterer Satz von Patentansprüchen (Art. 167(2)a)) Additional set of claims (Art 167(2)a)) Série supplémentaire de revendications (art. 167(2)a))	AUCL (1)	38	AT	Zahl der Patentansprüche / Number of Nombre de revendications
	AUCL (3)		ES	
	AUCL (4)		GR	
Zur Veröffentlichung mit der Zusammenfassung wird vorgeschlagen Abbildung Nr. / With the abstract it is proposed to publish figure No. / Il est proposé de publier avec l'abrégé la figure n°		39	Z	Nummer / Number / Numéro
Zusätzliche Abschrift(en) der im europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt / Additional copy(ies) of the documents cited in the European search report is (are) requested / Prière de fournir une (des) copie(s) supplémentaire(s) des documents cités dans le rapport de recherche européenne		40	<input type="text"/>	Anzahl der zusätzlichen Sätze von Abschriften Number of additional sets of copies Nombre de jeux supplémentaires de copies
Es wird die Rückerstattung der Recherchegebühr gemäß Art. 10 GebO beantragt / Refund of the search fee is requested pursuant to Article 10 of the Rules relating to Fees / Le remboursement de la taxe de recherche est demandé en vertu de l'article 10 du règlement relatif aux taxes		41	<input type="text"/>	
Eine Kopie des Recherchenberichts ist beigelegt / A copy of the search report is attached / Une copie du rapport de recherche est jointe		42	<input type="text"/>	
Eventuelle Rückzahlungen auf nebenstehendes beim EPA geführte laufende Konto / Reimbursement, if any, to EPO deposit account opposite / Remboursements éventuels à effectuer sur le compte courant ci-contre ouvert près l'OEB	DEPA	43	Nummer / Number / Numéro 28050100	Kontoinhaber / Account holder / Titulaire du compte THE WELLCOME FOUNDATION LIMITED
Die vorgeschriebene Liste über die diesem Antrag beigelegten Unter- lagen ergibt sich aus der vorberei- teten Empfangsbescheinigung (Seite 5 dieses Antrages)	The prescribed list of documents enclosed with this request is shown on the prepared receipt (page 5 of this request)	44	La liste prescrite des documents joints à cette requête figure sur le récépissé préétabli (page 5 de la présente requête)	
Unterschrift(en) des (der) Anmelders(s) oder Vertreter(s) / Signature(s) of applicant(s) or representative(s) / Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)		45		Für Angestellte nach Artikel 133 (3) Satz 1 mit allgemeiner Vollmacht / For employees under Article 133 (3), 1st sentence, having a general authorisation / Pour les employés mentionnés à l'article 133, paragraphe 3, 1 ^{re} phrase, munis d'un pouvoir général Nr. / No. / n°:
Ort / Place / Lieu	BECKENHAM, KENT, ENGLAND			
Datum / Date	8 OCTOBER 1991			
 STOTT, MICHAEL JOHN				
Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature, in case of legal persons, the position of the signer within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. Si s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire				

PA1195

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

(Liste der diesem Antrag beigefügten Unterlagen)

(Checklist of enclosed documents)

(Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents désignés ci-dessous

Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA). Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen. / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA). Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé de documents, ce récépissé est réputé être la notification visée à la règle 24(4). Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.

MR M J STOTT
THE WELLCOME RESEARCH LABORATORIES
LANGLEY COURT
BECKENHAM
KENT BR3 3BS
ENGLAND

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Datum / Date

THE PATENT OFFICE

17 OCT 1991

NEWPORT

Unterschrift / Amtsstempel / Signature / Official stamp / Signature / Cachet officiel

Anmeldenummer / Application No. / N° de la demande

91309595.6

Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))

DREC

17 OCT 1991

Zeichen des Anmelders / Vertreters / Applicant's / Representative's ref. / Référence du demandeur ou du mandataire

AREF

PA1195 / MJS

Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: /
Seulement après le dépôt de la demande auprès d'un service national:

Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))

RENA

A. Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité

46

Stückzahl
Number of copies
Nombre
d'exemplairesBlattzahl* eines Stücks
Number of sheets*
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exemplaireGesamtzahl
der Abbildungen
Total number of figures
Nombre total
de figures

1. Beschreibung / Description

3

28

2. Patentansprüche / Claim(s) / Revendication(s)

3

3

3. Ggf. unterschiedliche Patentansprüche (Art. 167(2)a) / Any different claims (Art. 167(2)a) / Le cas échéant, revendications différentes (art. 167(2)a)

-

-

4. Zeichnung(en) / Drawing(s) / Dessin(s)

DRAW 1 #

3

5

5. Zusammenfassung / Abstract / Abrégé

3

1

6. Übersetzung der Anmeldungsunterlagen / Translation of the application documents / Traduction des pièces de la demande

1

7. Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité

8. Übersetzung des (der) Prioritätsbelegs (belege) / Translation of priority document(s) / Traduction du (des) document(s) de priorité

B. Der Anmeldung in der eingereichten Fassung liegen folgende Schriftstücke bei: /
This application as filed is accompanied by the items below: /
A la présente demande sont annexés les documents suivants:

47

1. Einzelvollmacht / Specific authorisation / Pouvoir particulier

☐

2. Allgemeine Vollmacht / General authorisation / Pouvoir général

☐

3. Erfindernennung / Designation of inventor / Désignation de l'inventeur

☒

4. Früherer Recherchenbericht / Earlier search report / Rapport de recherche antérieure

☐

5. Gebührenzahlungsvordruck (EPA Form 1010) / Voucher for the settlement of fees (EPO Form 1010) / Bordereau de règlement de taxes (OEB Form 1010)

☒

6. Scheck (ausgeschlossen bei Einreichung bei den nationalen Behörden) / Cheque (not when filing with national authorities) / Chèque (pas de chèque en cas de dépôt auprès des services nationaux)

☐

7. Zusatzblatt / Additional sheet / Feuille additionnelle

☐

8. Sonstige Unterlagen (bitte hier spezifizieren) / Other (please specify here) / Autres documents (veuillez préciser ici)

☐

C. Kopien dieser Empfangsbescheinigung / Copies of this receipt for documents / Copies du présent récépissé de documents

48

4

Anzahl der Kopien / Number of copies / Nombre de copies

* Die Richtigkeit der Angabe der Blattzahl wurde bei Eingang nicht geprüft / No check was made on receipt that the number of sheets indicated was correct / L'exactitude du nombre de feuilles n'a pas été contrôlée lors du dépôt

PA1195

ERFINDERNENNUNG / DESIGNATION OF INVENTOR / DESIGNATION DE L'INVENTEUR 2

(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen/max. 15 spaces/
15 caractères au maximum)

PA1195 ✓

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)
In respect of the above European patent application I (we), the undersigned
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)

THE WELLCOME FOUNDATION LIMITED ✓

als Erfinder:
do hereby designate as inventor(s):
désigne(nt) en tant qu'inventeur(s):

PAGE, MARTIN JOHN of Langley Court, Beckenham, Kent BR3 3BS, England

GB # 14

☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) /
(les autres inventeurs sont mentionnés sur une feuille supplémentaire)

☒ Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt³
The applicant(s) has (have) acquired the right to the European patent³
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen³

☐ gemäß Vertrag vom _____
under an agreement dated _____
par contrat en date du _____

☒ als Arbeitgeber
as employer(s)
en qualité d'employeur(s)

☐ durch Erbfolge
as successor(s) in title
par transfert successoral

☒ Für jeden Erfinder, der nicht zugleich Anmelder ist, ist eine Kopie der Erfindernennung beigelegt⁴.
A copy of the designation of inventor is enclosed for each inventor who is not also an applicant⁴.
Une copie de la désignation de l'inventeur à l'intention de chaque inventeur, sauf s'il est en même temps demandeur, est jointe à la présente⁴.

Ort/Place/Lieu BECKENHAM, KENT, ENGLAND

Datum/Date

8 October 1991

Unterschrift(en) des (der) Anmelders (Anmelder) oder Vertreters (Vertreter)
Signature(s) of applicant(s) or representative(s)
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

STOTT MICHAEL JOHN ✓

Bitte Namen des (der) Unterzeichneten mit Schreibmaschine wiederholen / Please supplement signature(s) by typewritten name(s) / Prière de dactylographier le(s)
nom(s) du (des) signataire(s) au-dessous de la signature -

Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

5

ANTIBODY PRODUCTION

The present invention relates to Chinese hamster ovary (CHO) cell lines, to the production of proteins, in particular antibodies from such cell lines, also to antibodies having CHO glycosylation.

Antibodies or immunoglobulins are proteinaceous bifunctional molecules. One region which is highly variable between different antibodies is responsible for binding to an antigen (Fab region), for example the many different infectious agents that the body may encounter, whilst the second, constant region (or Fc region) is responsible for binding to the Fc receptors of cells and also activates complement. In this way, antibodies represent a vital component of the immune response of mammals in destroying foreign microorganisms and viruses.

An antibody molecule is composed of two light chains and two heavy chains that are held together by interchain disulphide bonds. Each light chain is linked to a heavy chain by disulphide bonds and the two heavy chains are linked to each other by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains, and each light chain has a variable domain at one end and a constant domain at the other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The remaining constant domains of the heavy chains are aligned with each other. The constant domains in the light and heavy chains are not involved directly in binding the antibody to the antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. They have the same general structure with each domain comprising a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet

conformation and the CDRs form loops connecting, and in some cases comprising part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

The immunisation of an animal with an antigen results in the production of different antibodies with different specificities and affinities. An antiserum obtained from the immunised animal will, therefore, be heterogeneous and contain a pool of antibodies produced by many different lymphocyte clones. Antibodies thus obtained are referred to as polyclonal antibodies and this polyclonal nature has been a major drawback in the use of antibodies in diagnostic assays and in therapeutic applications.

A major step forward occurred in 1975 when Kohler and Milstein (Nature, 1975, 256, 495-497) reported the successful fusion of spleen cells from mice immunized with an antigen with cells of a murine myeloma line. The resulting hybrid cells, termed hybridomas, have the properties of antibody production derived from spleen cells and of continuous growth derived from the myeloma cells. Each hybridoma synthesizes and secretes a single antibody to a particular determinant of the original antigen. To ensure that all cells in a culture are identical, i.e. that they contain the genetic information required for the synthesis of a unique antibody species, the hybridomas resulting from cell fusion are cloned and subcloned. In this way, the cloned hybridomas produce homogeneous or monoclonal antibodies.

The advantages of hybridoma technology are profound. Because many hybrids arising from each spleen are screened for their potential to produce antibodies to the antigen of interest and only a few are selected, it is possible to immunize with impure antigens and yet obtain specific antibodies. The immortality of the cell line assures that an unlimited supply of a homogeneous, well-characterised antibody is available for use in a variety of applications including in particular diagnosis and immunotherapy of pathological disorders.

Unfortunately, the usefulness of such monoclonal antibodies in a clinical setting can be severely hampered by the development of human anti-mouse antibodies - an anti-globulin response - which may interfere with therapy or cause allergic or immune complex hypersensitivity.

When, for example, murine (or ratine) monoclonal antibodies are used in human therapy, the induction of an human anti-mouse antibody response is due to the murine origin of the constant domains and four framework regions. This problem has therefore been addressed by the development of antibodies of two basic types: The first type, referred to as "chimeric antibodies", is where the murine constant domains only are replaced by equivalent domains of human origin (Morrison *et al.*, *P.N.A.S.*, 1984, 81, 6851-6855; Boulianne *et al.*, *Nature*, 1985, 314, 268-270; and Neuberger *et al.*, *Nature*, 1985, 314, 268-270). The second type is where the murine constant domains and the murine framework regions are all replaced by equivalent domains and regions of human origin. This second type of antibody is referred to as a humanised or CDR-grafted antibody (Jones *et al.*, *Nature*, 1986, 321, 522-525; and Riechmann *et al.*, *Nature*, 1988, 332, 323-327). A human antibody would of course avoid the need for "humanisation", however cell lines which secrete human antibodies are very unstable and have generally proven unsuitable for commercial scale production.

To generate sufficient quantities of antibody for full clinical use it is desirable to employ an efficient recombinant expression system. Since myeloma cells represent a natural host specialized for antibody production and secretion, cell lines derived from these have been used for the expression of recombinant antibodies. Often, complex vector design, based around immunoglobulin gene regulatory elements, is required, and final expression levels have been reported which are highly variable (Winter *et al.*, *Nature*, 1988, 332, 323-327; Weidle *et al.*, *Gene*, 1987, 60, 205-216; Nakatani *et al.*, *Bio/Technology*, 1989, 7, 805-810; and Gillies *et al.*, *Bio/Technology*, 1989, 7, 799-804).

An alternative mammalian expression system is that offered by the use of dihydrofolate reductase (dhfr) deficient Chinese hamster ovary (CHO) cells. The use of these cells has enabled the production of large quantities of several therapeutic proteins for research and clinical use (Kaufman *et al*, Mol.Cell.Biol, 1985, 5, 1750-1759; and Zettlmeissl *et al*, Bio/Technology, 1987, 5, 720-725). There are, however, very few instances of the use of these cells for the expression of antibodies and the levels of expression of murine antibodies reported to date are low - of the order of 0.01-0.1 μ g/ml (Weidle *et al*, Gene, 1987, 51, 21-29; and Feys *et al*, Int.J.Cancer, 1988, 2, 26-27).

A process has now been developed that enables the balanced expression of the light and heavy chains of an antibody from CHO cells. Balanced expression is desirable given that the light and heavy chains are linked together in the antibody molecule in equimolar proportions. This process allows the antibody to be obtained in functional form and to be secreted in good yields. Thus the process enables sufficient quantities of functional antibody to be obtained for use in the immunotherapy of pathological disorders.

The invention therefore provides a CHO cell line capable of producing antibody, the cell line having been co-transfected with a vector capable of expressing the light chain of the antibody and a vector capable of expressing the heavy chain of the antibody wherein the vectors contain independently selectable markers.

The present invention further provides a CHO cell line capable of producing a human antibody or an altered antibody, the cell line having been co-transfected with a vector containing cDNA encoding the light chain of the antibody and a vector containing cDNA encoding the heavy chain of the antibody said vectors capable of expressing the light and heavy chains of the antibody. The vectors may advantageously contain independently selectable markers. Hereafter, reference to the markers includes the singular and vice versa.

The cell line of the present invention is capable of producing all kinds of antibodies that generally comprise equimolar proportions of light and heavy chains. The invention therefore includes human antibodies wherein the amino acid sequences of the heavy and light chains are homologous with those sequences of antibodies produced by human lymphocytes in vivo or in vitro by hybridomas. Also included in the invention are altered antibodies such as hybrid antibodies in which the heavy and light chains are homologous to a natural antibody but are combined in a way that would not occur naturally. For example, a bispecific antibody has antigen binding sites specific to more than one antigen. The constant region of the antibody may relate to one or other of the antigen-binding regions or may be from a further antibody. Altered antibodies, such as chimaeric antibodies have variable regions from one antibody and constant regions from another. Thus, chimaeric antibodies may be species/species chimaeras or class/class chimaeras. Such chimaeric antibodies may have one or more further modifications to improve antigen binding ability or to alter effector functioning. Another form of altered antibody is a humanised or CDR-grafted antibody including a composite antibody, wherein parts of the hypervariable regions in addition to the CDRs are transferred to the human framework. Additional amino acids in the framework or constant regions of such antibodies may be altered. Included in the definition of altered antibody are Fab fragments which are roughly equivalent to the Y branch portions of the heavy and light chains; these may be included incomplete fragments or fragments including part of the Fc region. Thus, within the scope of the invention is included, any altered antibody in which the amino acid sequence is not one which exists in nature.

The cell line of the invention is preferentially employed for the production of altered antibodies most preferably chimaeric antibodies or CDR-grafted antibodies. Particular examples of these include antibodies against T cell markers such as CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD11b, CD18, CD19, CD25, CD45 and CDw52 and especially CDR grafted antibodies against the CDw52 antigen, such as Campath-1H

(Campath is a Trademark of the Wellcome Foundation Ltd) described in EP 328404. Further examples include CDR-grafted antibodies against various cancer cell marker antigens such as CD33 and CD38.

After co-transfection into recipient CHO cells, the resulting colonies may be selected using both markers. Colonies exhibiting the dual phenotype are generally capable of co-expressing both the light and heavy chains. The selectable markers may or may not be of a dominant nature. Examples of selectable markers for use in co-transfection include adenosine deaminase (Kaufman *et al*, P.N.A.S., 1989, 83, 3136-40), asparagine synthetase (Cartier *et al*, Mol. Cell Biol., 1987, 7, 1623-28), E. coli trpB gene and Salmonella hisD gene (Hartman *et al*, P.N.A.S., 1988, 85, 8407-51), M2 mouse ribonucleotide reductase (Thelander *et al*, EMBO J., 1989, 8, 2475-79), human multidrug resistance gene (Kane *et al*, Gene, 1989, 84, 439-446), glutamine synthetase (Bebbington *et al*, DNA Cloning, Vol III, 1987, Ed. D.M. Glover, 163-188, IRL Press), xanthine guanine phosphoribosyl transferase (gpt) (Mulligan *et al*, Science, 1980, 209, 1422-27), hygromycin B (Santerre *et al*, Gene, 1984, 30, 147-156), neomycin gene (Southern *et al*, J. Mol. Appl. Genet., 1982, 1, 327-341), and dihydrofolate reductase (Subramani *et al*, Mol. Cell Biol., 1981, 1, 854-864). One particularly preferred selectable marker is dhfr which is usually employed with a parental CHO cell line of the dhfr⁻ phenotype (Urlaub *et al*, P.N.A.S., 1980, 77, 4216-4220). Successfully co-transfected CHO cells will possess the dhfr⁺ phenotype and can readily be selected by culturing the colonies on media devoid of thymidine and hypoxanthine and optionally containing methotrexate (MTX). A preferred selectable marker for use with the other of the vectors is a dominant resistance marker, such as neomycin (neo). CHO cells successfully transfected with this marker can readily be selected by culturing the colonies on media containing the antibiotic, G418, otherwise known as Geneticin.

A second preferred system of selection and amplification is provided by the glutamine synthetase selectable marker or (GS system) which is described in WO87/04462. CHO cells which have been successfully transfected with the gene encoding the GS enzyme and the desired antibody gene can be selected by culturing colonies in media devoid of glutamine as described in PCT published application number WO87/04462.

At least one of the selectable markers preferably also provides the basis upon which the genes encoding the light and heavy chains may be amplified. In co-transfection of a CHO cell line, the vector DNAs are often integrated into the chromosome of the cell at the same locus. Thus, the use of only one of the selectable markers as the basis for amplification normally results in a parallel increase in the copy number of both genes. One particularly preferred selectable marker for use in this way is dhfr which enables the desired amplification to be obtained through the use of increasing concentrations of MTX. A second preferred selectable marker is GS which allows amplification by the addition of methionine sulphoximine (MSX).

The selectable markers are of course under the control of regulatory elements of DNA so as to provide for their expression. In the case of the use of dhfr as a selectable marker, the regulatory elements are preferably of a viral source, such as from DNA tumour viruses. Particularly preferred are the use of an SV40 or adenovirus major late promoter. It is particularly advantageous in this regard to remove the enhancer element from the promoter thus effectively "crippling" it. This modification allows for increased levels of gene amplification at each concentration of methotrexate selection than would otherwise occur if a strong promoter was used. In the case of the use of neo as a selectable marker, an example of a suitable promoter is the mouse metallothionein promoter.

The light and heavy chain genes may constitute genomic DNA or, preferably, cDNA, and are cloned using procedures known in the art (Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis

et al, Cold Spring Harbor). The genes are also under the control of regulatory elements of DNA so as to provide for their expression. The use of the same regulatory elements for both chains is preferred so that their expression is substantially balanced. The regulatory elements may be of viral origin and examples include those mentioned above in conjunction with the expression of dhfr as a selectable marker. Another example is the use of the β -actin promoter and cognate- β -actin polyadenylation signal.

One or both of the vectors may also contain an SV40 origin of replication to allow for the vector constructs to be checked by rapid transient assay.

Construction of the expression vectors may be carried out in accordance with procedures known in the art (Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis et al, Cold Spring Harbor).

Co-transfection of the CHO cell line with the expression vectors may be carried out simply by using equimolar quantities of both vectors and standard transfection procedures, such as calcium phosphate precipitation or lipofectin. Selection of the desired co-transfected cell line may be carried out in accordance with standard procedures known for the particular selectable markers.

The present invention also provides a process for the production of an antibody which comprises culturing a CHO cell line of the present invention. Culture of the CHO cell line may be carried out in serum-containing or preferably serum and protein free media. In one preferred instance where the CHO cell line is a dhfr⁺ transformant, the medium preferably lacks hypoxanthine and/or thymidine and optionally contains MTX. Where a selectable marker is glutamine synthetase the medium preferably lacks glutamine and optionally contains MSX. Expression of both chains in substantially equimolar proportions enables optimum yields of functional antibody to be

obtained. The two chains assemble within the cell and are then secreted into the culture medium as functional antibody. The resulting antibody may be purified and formulated in accordance with standard procedures.

Antibodies are glycoproteins containing between 3 and 12% carbohydrate. The carbohydrate units are transferred to acceptor sites on the antibody chains after the heavy and light chains have combined. The major carbohydrate units are attached to amino acid residues of the constant region of the antibody. Carbohydrate is also known to attach to the antigen binding sites of some antibodies and may affect the antibody-binding characteristics by limiting access of the antigen to the antibody binding site. There are a number of roles associated with the carbohydrate units. They may affect overall solubility and the rate of catabolism of the antibody. It is also known that carbohydrate is necessary for cellular secretion of some antibody chains. It has been demonstrated that glycosylation of the constant region plays a vital role in the effector functioning of an antibody; without this glycosylation in its correct configuration, the antibody may be able to bind to the antigen but may not be able to bind for example to macrophages, helper and suppressor cells or complement, to carry out its role of blocking or lysing the cell to which it is bound.

It has now been found that antibody glycosylated by CHO cells maintains antigen binding capability and effector functionality. This has been demonstrated in in vitro complement lysis assays and in vivo in a human patient.

The invention therefore provides an antibody having CHO glycosylation. Such antibodies may be natural, such as human antibodies, altered antibodies for example hybrid antibodies or bispecific antibodies, chimaeric or CDR-grafted antibodies, including Fab fragments.

The CHO glycosylation may be associated with the antigen binding site or other parts of the variable domain. It may alternatively or additionally be associated with the constant region. The glycosylated antibody is prepared by expression of the antibody genes in a suitably engineered CHO cell followed by recovery and if necessary, purification of the antibody from the cell culture medium.

CHO glycosylated antibodies are useful in medical therapy for treating numerous human disorders, generally as immunosuppressives more particularly for example T-cell mediated disorders including severe vasculitis, rheumatoid arthritis, systemic lupis, also autoimmune disorders such as multiple sclerosis, graft vs host disease, psoriasis, juvenile onset diabetes, Sjogrens' disease, thyroid disease, myasthenia gravis, transplant rejection and asthma. These antibodies are also useful in treating cancer such as Non-Hodgkins lymphoma, multiple myeloma, and infectious diseases such as HIV and herpes.

The invention therefore provides the use of CHO glycosylated antibodies in the manufacture of a medicament for the treatment of any of the aforementioned disorders. Also provided is a method of treating a human being having any such a disorder comprising administering to said individual a therapeutically effective amount of a CHO glycosylated antibody.

The dosages of such antibodies will vary with the condition being treated and the recipient of the treatment, but will be in the range 1 to about 1 00 mg for an adult patient preferably 1 - 10 mg usually administered daily for a period between 1 and 30 days. A two part dosing regime may be preferable wherein 1 - 5 mg are administered for 5 - 10 days followed by 6 - 15 mg for a further 5 - 10 days.

Also included within the invention are formulations containing CHO glycosylated antibody. Such formulations preferably include, in addition to antibody, a physiologically acceptable diluent or carrier

possibly in admixture with other agents such as other antibodies or an antibiotic. Suitable carriers include but are not limited to physiological saline, phosphate buffered saline, phosphate buffered saline glucose and buffered saline. Alternatively, the antibody may be lyophilised (freeze dried) and reconstituted for use when needed by the addition of an aqueous buffered solution as described above. Routes of administration are routinely parenteral including intravenous, intramuscular, subcutaneous and intraperitoneal injection or delivery.

The accompanying drawings show:

Figure 1

(a) the pLD9 construct containing expression cassettes for the 'crippled' dhfr selection/amplification marker and the Campath-1H light chain cDNA. The small box with the dashed arrow is the weakened SV40 promoter; the larger dotted box with an arrow is the β -actin promoter; polyA refers to respectively sourced polyadenylation and termination signals; the small box with ori contains the SV40 origin of replication;

(b) the pNH316 construct containing expression cassettes for the neomycin selection marker and the Campath-1H heavy chain cDNA. The box with an arrow and MT refers to the mouse metallothionein promoter. Restriction sites indicated are:- H, HindIII; Bg, BglII; B, BamHI; R1, EcoRI.

Figure 2

Comparative determinations of the rate of Campath-1H synthesis in confluent A39 cells over 4 consecutive days. Following the [35 S] methionine pulse period, equal aliquots of cells (C) and culture medium (M) were immuno-precipitated and separated by SDS-PAGE. The

position of the Campath-1H heavy and light chains are indicated (H and L arrows). There was some loss of material for the day 3 cell sample.

Figure 3

A pulse-chase experiment to determine the rate of secretion and distribution of radiolabelled Campath-1H in A39 cells. Confluent cells were pulsed with [³⁵S]-methionine for 6 hours, then fresh medium containing an excess of unlabelled methionine was added. Equal aliquots of cells and culture medium were taken at the indicated time points (in hours following the end of the pulse period) and treated as described in the legend of Figure 2. The samples for the 48 and 72 hour medium time points were run on a different gel to the 6 and 24 hour points, and the tracks are only lined up relative to the position of the heavy (H) chain.

Figure 4

Shows growth of ClH 3D11* 44 in WCM5 (protein-free medium) in a 1 litre fermenter measured as cell count/ml over 90 days.

Figure 5

Shows antibody production from ClH 3D* 44 cells in WCM5 in a 1 litre fermenter measured as micrograms of antibody/ml over 80 days.

The following Examples are provided purely for illustration of the present invention.

EXAMPLE 1: Cloning of the Heavy and Light Chain cDNAs for Campath-1H

The complementarity determining regions from the rat Campath-1G monoclonal were originally grafted directly into genomic human heavy and light chain frameworks (Winter *et al*, *Nature*, 1988, 322, 323-327). These constructs were engineered for expression in the myeloma cell

line YO and resulted in yields of Campath-1H of up to 5µg/ml following 10-14 days in culture (Hale *et al*, Tissue Antigens, 1990, 35, 118-127 and Winter *et al*, Nature, 1988, 322, 323-327). The myeloma cell line TF57 (Hale *et al*, *ibid*.) was used to generate size selected cDNA fractions of 0.9-1.2kb and 1.4-1.7kb for the light and heavy chain cDNAs respectively. These were used to make EcoRI linker cDNA libraries in λgt10. All procedures were as described by Huynh *et al* (DNA Cloning, Vol I: A Practical Approach, 1984, Glover, D(Editor), IRL Press, Oxford). The libraries were screened using [³²P] nick translated probes specific for the variable regions to isolate full length cDNA clones. For the light chain cDNA, the 5' untranslated leader was removed up to position -32 using Bal-31 exonuclease and a HindIII linker added. For the 3' end, use was made of a unique SacI site 47bp upstream of the stop codon. A SacI-HindIII oligonucleotide pair was used to regenerate this sequence and position the HindIII site immediately after the stop codon. For the 5' end of the heavy chain cDNA, the unique NcoI site overlapping the ATG start codon was used to re-build a 29bp untranslated leader, identical to that of the light chain, using a HindIII-NcoI oligonucleotide pair. At the 3' end, the unique NaeI site 12bp downstream of the stop codon was converted into a HindIII site using linkers.

EXAMPLE 2: Construction of Vectors:

The human β-actin promoter was excised from pHβAPr-3-neo (which corresponds to pHβAPr-1-neo (Gunning *et al*, P.N.A.S., 1987, 84, 483-35) except that the SV40 polyadenylation/termination signal has been replaced with the respective human β-actin signals) as a 2860 bp PvuII-HindIII fragment, in which the PvuII site was subsequently converted to a BglII site using linkers. To isolate the human β-actin polyadenylation and termination signals from pHβAPr-3-neo, an SphI site 1.4kb downstream of the unique HindIII site was converted to a BamHI site using linkers. The basal dhfr vector called p104, was constructed as follows. The SphI site at position -128 in the SV40 promoter in pSV2dhfr (Subramani *et al*, Mol.Cell.Biol., 1981, 1,

854-864) was converted into a Sall site to remove all enhancer elements from the promoter. The weakened dhfr expression unit was then subcloned as a Sall-BamHI fragment into the homologous sites in pSV0d (Mellon *et al.*, *Cell*, 1981, 27, 279-288).

To construct pLD9, the p104 vector was digested with BamHI, phosphatased, and ligated with three other fragments consisting of the BglII-HindIII β -actin promoter, the HindIII Campath-1H light chain cDNA and the HindIII-BamHI β -actin polyA/termination signals. To construct pNH316, the construct pdBPV-MMTneo (Law *et al.*, *Mol. Cell. Biol.*, 1983, 3, 2110-2115) was digested with BamHI, phosphatased, and the fragment containing the neomycin gene isolated following separation on an agarose gel. This was ligated to the two β -actin fragments and the Campath-1H heavy chain cDNA. The constructs, pLD9 and pNH316 are depicted in Figure 1.

EXAMPLE 3: Expression of Campath-1H in CHO Cells:

The dhfr⁻ CHO cell line DUK-B11 (Urlaub *et al.*, *P.N.A.S.*, 1980, 77, 4216-4220) was grown in Iscove's MEM supplemented with 10% fetal bovine serum, and 4 μ g/ml each of hypoxanthine and thymidine. 10 μ g of pLD9 and pNH316 was co-precipitated onto cells using the calcium phosphate method, (Gorman *et al.*, *DNA Cloning*, 1985, Vol II, 143-190, Academic Press, N.Y.) and selected for the double phenotype of dhfr⁺/neo resistance by using the medium above except that 10% dialysed serum was used, the hypoxanthine/thymidine were omitted, and G418 (Gibco) was included at 500 μ g/ml. In some experiments MTX was included directly in the first round selection for dhfr⁺ transformants. Several hundred resistant colonies were pooled and assayed for the production of Campath-1H antibody in the culture medium. The average yield was 0.5 μ g/ml for non-amplified first round transformants.

Each pooled cell population was then cultured in the presence of 10⁻⁷M MTX, and after two weeks, resistant colonies were again pooled and

titred for Campath-1H production. There was a considerable increase in yield of up to 80-fold (Table 1). These cells were dilution cloned, screened for Campath-1H yield, and two high producer lines isolated, called A37 and 3D9 (Table 1). These were both amplified further in the presence of 10^{-6} M MTX, then dilution cloned and screened as above. The increase in expression at this second, and final, amplification stage was not so dramatic as seen previously; nevertheless, when re-fed at confluence and left for a further 4-days, the cell lines A39 and 3D11 were capable of producing up to 200 μ g/ml of Campath-1H.

TABLE 1

Expression Levels of Campath-1H using Stepwise Amplification

Construct	Selection stage	Accumulated Campath-1H (μ g/ml)
pLD9 + pNH316	dhfr ⁺ /neo basal pool	0.5
	10^{-7} M MTX amplified pool	18-40
	Cell lines A37 and 3D9	40
	10^{-6} M MTX amplified pool	60-90
	Cell line A39	100
	Cell line 3D11	150-200

Legend to Table

Cells were allowed to reach confluence in a T-175 tissue culture flask, then re-fed with fresh 50ml of tissue culture medium and left for a further 4 days. The Campath-1H antibody that had accumulated in the medium during this period was measured by ELISA. Total cell counts on the day of assay were usually 2.5×10^7 . The yield from the 3D11 cell line reflects a productivity of $100\mu\text{g}/10^6$ cells/day.

The co-transfection vectors pLD9 and pNH316 were further employed to evaluate an alternative amplification strategy to the one described above. The dhfr⁻ CHO cells were co-transfected as usual, and two days later split directly into a series of flasks containing G418 (for neomycin selection) and increasing concentrations of MTX ranging from 3×10^{-9} M to 10^{-7} M. Following two weeks of this selection, the number of resistant colonies were counted and pooled for each flask. When the cell populations had stabilized, they were assayed for Campath-1H antibody titres and the results are shown in Table 2. As the MTX level was increased, there was a marked decrease in the number of surviving dhfr⁺ colonies, but they expressed proportionately more Campath-1H. Thus, in a one step direct selection at high concentrations of MTX, it is possible to isolate cell populations which produce up to 60-fold increase in antibody yield compared to cell populations selected for basal dhfr levels.

TABLE 2

Expression Levels of Campath-1H using Direct Selection

Selection (M MTX)	dhfr ⁺ colonies	Accumulated	
		Campath-1H	($\mu\text{g}/\text{ml}$)
No MTX	500		0.5

3×10^{-9}	40	2
10^{-8}	5	7
3×10^{-8}	5	30
10^{-7}	-	-

Legend to Table

Colonies at each MTX selection stage were pooled and assayed as described in the legend of Table 1.

This selection procedure was repeated following another co-transfection of cells, and in this instance, the entire population was selected in medium containing G418 and 3×10^{-8} M MTX. This generated a larger pool of resistant colonies which were subsequently pooled and re-amplified twice more using MTX concentrations of 6×10^{-7} M, then 3×10^{-6} M. At this stage, the cells were dilution cloned and screened for Campath-1H levels. The two highest producer cell lines isolated were capable of producing antibody levels up to 100-150 µg/ml and were designated as lines 4F11 and 5E10.

The growth rates of these cell lines, and the A39/3D11 lines described above, were considerably slower than the parental non-transformed dhfr⁻ CHO cells. This is usually a common feature of these cells once they have been engineered to express high quantities of a product gene. The yields from the 5E10 and 4F11 cell lines proved to be quite variable over time, and the latter appeared to have only a limited passage life lasting about 3 weeks before entering crisis and death. This instability was not evident at all in the other cell lines, although in general, the lines isolated from the second amplification procedure, including 5E10, were usually more fickle to culture. Of all the lines, the 3D11 coupled good growth and stability with high

Campath-1H yields. To ensure the propagation of these features, the 3D11 cell line was dilution cloned once more to generate the 3D11* line and this similarly produced Campath-1H yields up to 200µg/ml.

EXAMPLE 4: Growth of and Production from ClH 3D11* 44 in WCM4

a) ClH 3D11* cells growing as a monolayer in Iscoves + 10% FBS Flow, non-essential amino acids, 10^{-6} M Methotrexate and antibiotics, were approximately 90% confluent. These cells were removed from the plastic with trypsin/versene, washed in Iscoves medium without supplements, centrifuged and resuspended at 5×10^4 /ml in WCM4 medium Table 3 + 0.25% peptone + 0.1% polyethylene glycol (PEG) 10,000 + 0.5% fetal bovine serum (FBS) without methotrexate (MTX).

TABLE 3

Formulation for medium WCM4.

Iscoves modification of DMEM without BSA, transferrin and lecithin. Available from GIBCO Ltd., Unit 4, Cowley Mill Td. Est., Uxbridge UB8 27G. Similar to published medium (Iscoves and Melcher (1978) J. Exp. Med. 1. 47, 923) without the bovine serum albumin, pure human transferrin, or soyabean lecithin.

+ 5 ml/litre	200mM L glutamine
+ 50 mg/litre	L proline
+ 50 mg/litre	L threonine
+ 50 mg/litre	L methionine
+ 50 mg/litre	L cysteine
+ 50 mg/litre	L tyrosine
+ 25 mg.litre	ascorbic acid
+ 0.062 mg.litre	vitamin B6
+ 1.36 mg.litre	vitamin B12
+ 0.2 mg/litre	lipoic acid

+	0.088 mg/litre	methyl linoleate
+	1 μ M	methotrexate
+	1 mg/litre	FeSO ₄
+	1 mg/litre	ZnSO ₄
+	0.0025 mg/litre	CuSO ₄
+	5 mg/litre	recombinant insulin (Nucellin)
+	50,000 Iu/litre	polymyxin
+	20,000 Iu/litre	neomycin
+	0.16 mg/litre	putrescine-2 HCL.

Three 25cm² flasks were set up with 10ml of cell suspension + hypoxanthine (H)-, thymidine (T) or HT. These flasks were incubated at 36.5°C in 5% CO₂ incubator.

After six days, the flasks were pooled and added to an equal volume of WCM4 + MTX without peptone or PEG, and were transferred to a 75cm² flask.

These cells were used to seed a 500ml Techner spinner, incubated at 36.5°C spinning at 40 rpm. Cells continued growing serum free for a period of over five months and although it was found that the cells needed a period of adaptation, the growth rate and viability steadily improved. The population doubling time was calculated to be 73.1 hours over approximately 7 weeks; this decreased to 47.4 hours over the subsequent 20 days then stabilised. Antibody secretion remained high at levels in excess of 60 μ g/ml. It was determined that the gene copy number in these cells did not decrease according to band intensity using Northern blot analysis.

In fermenters, these cells produced antibody in excess of 70 μ g/ml and regularly achieve levels of 100 μ g/ml or more. These cells are denoted ClH 3D11* 44.

b) Cells from a) above which had been growing serum-free for over 2 months were transferred to a SGi 1 litre fermenter with a stainless

steel angled paddle turning at 70rpm. The temperature was set at 37°C, dO₂ at 10% and pH control to 7-7.2. The fermenter was seeded on day 0 with 0.22 x 10⁶ cells/ml in WCM4 (Table 3) with 0.1% polyethylene glycol (PEG) 10,000 and 0.25% soy peptone, and was top gassed with O₂. The cells were routinely passaged using fresh medium and a split rate typically between 1 to 2 and 1 to 4.

On day 33 the top gassing was replaced with deep sparging which is can be expected to cause more physical damage to the cells.

On day 50 onwards WCM5 (Table 4) was used together with peptone and PEG instead of WCM4.

TABLE 4

Formulation for Medium WCM5

Iscoves modification of DMEM without BSA, transferrin or lecithin (see Table 3).

+	5 ml/litre	200mM L glutamine
+	50 mg/litre	L proline
+	50 mg/litre	L threonine
+	50 mg/litre	L methionine
+	50 mg/litre	L cysteine
+	50 mg/litre	L tyrosine
+	25 mg/litre	L ascorbic acid
+	0.062 mg.litre	Vitamin B6
+	1.36 mg.litre	Vitamin B12
+	2 mg/litre	Ferric citrate
+	1 mg/litre	Zinc sulphate
+	0.0025 mg.lit	Copper sulphate
+	50,000 IU/litre	Polymyxin
+	20,000 IU/litre	Neomycin
+	3 µl/litre	Ethanolamine

+	0.16 mg/litre	Putrescine
+	5 mg/litre	Recombinant Insulin (Nucellin)

On day 53 the PEG was replaced with 0.1% pluronic F68. The resulting growth and antibody levels achieved are shown in the attached graphs (Figs 4 and 5), and demonstrate the capacity of the invention to allow protein-free production of antibody in excess of 100µg/ml in fermenters.

EXAMPLE 5: Analysis of the Rate of Campath-1H Synthesis and Secretion from CHO Cells:

During the course of culturing the Campath-1H producing CHO cells of Example 3, it became clear that even when they reached confluence, antibody levels continued to accumulate, with time, in the culture medium. To determine whether this was possibly a consequence of intracellular accumulation coupled to slow secretion, the rates of Campath-1H synthesis and secretion were measured using A39 cells. These analyses were performed over 3-4 consecutive days on cells which were either in growth phase, or confluent stationary phase. For the cells in either growth state, the results were identical, and data is presented only for the immuno-precipitated radiolabelled Campath-1H produced from stationary cells.

The rate of antibody synthesis was measured by pulsing the cells for a short period with [S^{35}]-methionine on each of four consecutive days, and then examining the quantity, and distribution, of immuno-precipitated material. In Figure 2, it is clear that the rate of synthesis is equally high at all time points measured. Furthermore, even by the end of this short pulse, in each case, more than half of the newly synthesized Campath-1H is already present in the medium suggesting rapid secretion. This was confirmed by the data shown in Figure 3, in which parallel cells were similarly pulsed, and the distribution of the radiolabelled Campath-1H chased over a three day period. Within 24 hours, virtually all of the cellular

radiolabelled antibody has been chased into the medium, where it remained stable for the duration of the experiment. This demonstrates that even when the recombinant CHO cells remain stationary for long periods, the rates of Campath-1H synthesis and secretion are not diminished.

Campath-1H ELISA assay. Microtiter plates were coated with anti-human IgG and incubated with the assay sample (in culture medium). Antibody detection was visualized by using an anti-human gamma chain specific peroxidase conjugate.

Analysis of rates of Campath-1H synthesis and secretion. Cells from Example 3 were grown to confluence in 3cm tissue culture wells, then incubated for 30 minutes in methionine-free Dulbeccos's MEM containing 10% fetal calf serum. Following this, the cells were labelled in the presence of 120 μ Ci/ml [³⁵S] methionine (>800Ci/mmol; Amersham) for the appropriate time period, then either harvested and lysed in 500 μ l of NP-40 lysis buffer, or incubated further in normal growth medium. Then 125 μ l aliquots of cell lysate or culture medium were immuno-precipitated using goat anti-human IgG (heavy chain specific; Sigma) and 10% protein-A Sepharose (Pharmacia). Samples were then separated on 10% SDS-PAGE reducing gels according to Laemmli and the signals amplified with Enhance (NEN-Dupont). The dried gels were then autoradiographed overnight.

Biological assays for functional CHO-glycosylated Campath 1H

Complement lysis assay for Campath 1H

The complement lysis assay is a measure of antibody function expressed as specific activity, determined by the ability of a CHO-glycosylated antibody of known concentration to bind to a pre-determined number of cells and effect cell lysis.

The assay is carried out on Campath 1H from Example 4 using Karpas 422 cells (established from B-cell non-Hodgkin lymphoma cell line - Dyer et al., (1990) Blood, 75 704-714) expressing Campath antigen on the cell surface. 1.2×10^7 cells were loaded with radiolabel by incubating for 2 hours at 37°C in a CO₂ incubator in the presence of 600µCi of 51Cr (sodium chromate).

5.3 ml of the loaded cells in medium (total volume 23.5ml), were added to 12.5ml of normal human serum and 150µl of the mixture were pipetted into the wells of a microtitre plate.

50µl samples of the final eluate from three purification runs were mixed with the cells and incubated for 30 minutes at 4°C followed by 90 minutes at 37°C. The culture was centrifuged at 2000 rpm for 5 minutes and the radioactivity in 100µl of cell supernatant was counted on a gamma counter. Complement lysis activity in Kilo Units/ml was calculated from a standard curve of a reference preparation (1000 Units/ml).

The results are set out in Table 5.

The concentration of Campath 1H in the 50µl samples of final eluate was estimated using samples in PBS pH 7.2 read on a spectrophotometer at 280nm. The results are expressed in Table 3 as optical density in mg/ml.

From this data the specific activity: KU/ml is determined.

OD

TABLE 5

<u>Sample</u>	<u>Complement lysis</u> <u>Kilo Units/ml</u>	<u>Protein Conc</u> <u>mg/ml</u>	<u>Specific</u> <u>Activity</u>
A	11.2	11.1	1.0
B	14.8	14.2	1.0
C	13.7	13.6	1.0

The results indicate that CHO-glycosylated Campath 1H is functional.

Treatment of an individual with CHO-glycosylated Campath 1H

An individual diagnosed as having severe T-cell mediated inflammation of the joints (immobilising polyarthrititis, pleuritis, abdominal pains) over five years requiring long periods of hospitalisation was treated with CHO derived Campath 1H from Example 4 using the following regime:

2mg per day over 6 days by intravenous injection

10 mg-per day over subsequent 6 days by intravenous injection.

During the second 6 day treatment there was a significant symptomatic improvement. By the end of the second period the joint inflammation was much improved and a skin abscess had cleared with antibiotic treatment. Thirty days after the end of the treatment the individual was discharged.

Approximately 9 months after the initial treatment, the individual suffered a relapse with multiple joint involvement. After initial testing for sensitivity with a low dose, the individual was given a further course of treatment with 10mg/day Campath 1H for 10 days with significant improvement.

EXAMPLE 6

EXPRESSION OF HUMANISED ANTI-CD4 ANTIBODY FROM CHO CELLS

Construction of the expression vector pBan1: modification of p342-12

The complementarily determining regions from a rat IgG2b raised against human CD4 (The New England Journal of Medicine 1990 323 : 250-254). were grafted onto human heavy and light chain frameworks (Winter et al, Nature, 1988, 322 323-327).

The cDNA encoding the humanised CD4 light chain was cloned into pLD9 [Page and Sydenham, M.A. 1991 Biotechnology 9 64-68]. The resulting plasmid was designated p2110. The humanised CD4 heavy chain was sequenced and cloned into a modified version of plasmid p342-12 [Law M-F., Byrne, J.C. and Hinley, P.M. 1983 Mol. Cell. Biol. 3 2110-2115]. Plasmid p342-12 was digested with BamH1 to remove the 7.4kbp fragment containing part of the BPV-1 genome. The backbone containing the β -lactamase gene and the neomycin resistance gene under the control of the mouse metallothionine promoter was purified and religated at the BamH1 site. This plasmid was digested with HinDIII, incubated with the large fragment of DNA polymerase I to remove the HinDIII site and then religated. The β -actin expression cassette, containing the β -actin promoter immediately upstream of a unique HinDIII site followed by the polyadenylation signal, was cloned into the BamHI site of the modified p342-12 plasmid to generate pBanI.

Plasmid pBanI, therefore, consisted of the neomycin resistance gene, the β -lactamase gene and the β -actin expression cassette containing the unique HinDIII site. The cDNA encoding the humanised heavy chain was cloned into this site and the resulting plasmid containing the correctly orientated insert was designated pBanCD4H. Thus, p2110 and pBanCD4H contained a different selectable marker and co-transfection into recipient dhfr- CHO cells would permit the direct selection and isolation of dhfr⁺/neo^r colonies. Cells exhibiting this phenotype should express functional antiCD4 antibody and could be amplified to elevate the antibody titres.

Expression of anti-CD4 antibody in CHO cells

a) Cell culture methods.

The dhfr- CHO line DUK-B11 [Urlaub, G. and Chasin, L.A. 1980 Proc.Natl.Acad.Sci.USA 77 4216-4220] was propagated in Iscoves MEM medium supplemented with 10% foetal bovine serum and 4 μ g each of hypoxanthine and thymidine (all Flow). After transfection,

transformants were selected in the medium described above except that the hypoxanthine/thymidine were omitted and dialysed foetal bovine serum was used. In addition, G418 was included at 500µg/ml. To induce spontaneous amplification of sequences containing and flanking the dhfr gene, MTX was added to a concentration of 0.1µM.

b) Transfection and amplification

The dhfr- CHO cell line DUK-B11 was co-transfected with 5µg of p2110 and 5µg of pBanCD4H using the transfectam reagent under the conditions recommended by the manufacturer. Transformants were selected for the $dhfr^+$ / neo^r phenotype as described above. Several hundreds of transformants were observed and pooled. Initial titres indicated that the first round basal transformants were secreting about 0.1µg/ml/day. This pooled population was then cultured in the presence of 0.1µM MTX for about 14 days. Resistant colonies were again pooled and assayed. Expression had increased some 100 fold, the pooled, amplified colonies producing about 10-12µg/ml/day. In order to obtain stable, clonal cell lines giving high antibody titres, the resistant pools were cloned by limiting dilution in 96-well plates. Fifty single colonies were identified and assayed and the four lines giving the highest titres propagated. This process of identifying highly expressing clones within the resistant population produced a line designated D419 which expressed the anti-CD4 antibody at about 20µg/ml/day.

Characterisation of $dhfr^+$ / neo^r cell lines

- i)- Determination of copy number and steady state transcription levels by slot blot analysis of DNA and RNA.

Whole cell RNA and DNA was prepared from the various stages of amplification as described by Maniatis *et al.* [1982 Molecular

Cloning. A Laboratory Manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York]. After fixing onto nitrocellulose filters, the nucleic acids were probed with [32 -P]- α ATP labelled DNA sequences of the heavy chain, the dhfr gene and the β -actin gene as a control "housekeeping" gene to eliminate artifacts due to loading errors.

Initially, the uncloned 0.1 μ M MTX amplified pool was compared to the first round unamplified transformants and the untransformed parental B11 cells, with the probes described. Accordingly, no DNA signal was detected in the parental line when probed with the heavy chain but a weak signal was detected for dhfr. This is due to the single, non-functional dhfr allele in the B11 cell line. As a result, no RNA signal was detected with either probe. In contrast, a strong signal was detected with both probes on RNA and DNA in the primary transformants which reflects the start of expression. A very significant increase in copy number and steady state levels of RNA of heavy chain and dhfr is observed in the uncloned amplified pool. This accurately correlates with the observed increase in expression. Steady state levels of β -actin RNA were consistent in all three lines examined.

A similar comparison was made between the four highest expressing cloned cell lines. A strong signal was detected on both the RNA and the DNA blots. However, although the DA19 line was expressing twice as much antibody as a line designated D423, this difference was not in either the copy number or steady state levels of RNA. There are two possible explanations for this observation; the first is that the DNA in the DA19 line has integrated at a site in the genome at which it is under the influence of an enhancer. However, this presumably would be reflected in elevated levels of RNA. The more likely explanation is that in the replication and duplication of the tandem arrays in the line D423, some of the copies of the dhfr/antibody cassette have undergone re-arrangement and are non-functional and

truncated. This is not uncommon since the site of integration of heterologous genes is often at breakpoints in the chromosomes such as telomeres which are known to be "hot spots" for such re-arrangements. This could be resolved by Northern and Southern analysis.

ii) Protein synthesis and secretion of anti-CD4 antibody in the D419 line.

The clonal D419 line was labelled with ^{35}S -methionine and cysteine and the intracellular and secreted antibody extracted by immunoprecipitation with appropriate antibodies. Following electrophoresis on reducing SDS-PAGE gels, the gels were dried and the signal detected by autoradiography.

It was clear from the result that both heavy and light chain are efficiently synthesised. Intracellularly, there need not be stoichiometry between heavy and light chains since the two associate as they pass through the secretory organelles. However, close stoichiometry is observed in the secreted material.

6

CLAIMS

1. A CHO cell-line capable of producing antibody, the cell-line having been co-transfected with a vector capable of expressing the light chain of the antibody and a vector capable of expressing the heavy chain of the antibody wherein the vectors contain independently selectable markers.
2. A CHO cell-line capable of producing a human antibody or an altered antibody, the cell-line having been co-transfected with a vector containing cDNA encoding the light chain of the antibody and a vector containing cDNA encoding the heavy chain of the antibody wherein said vectors are capable of expressing the heavy and light chains.
3. A CHO cell-line as claimed in Claim 2 wherein the vectors contain independently selectable markers.
4. A CHO cell-line as claimed in any of Claims 1-3 wherein one of the markers is dominant.
5. A CHO cell-line as claimed in any of Claims 1-4 wherein the markers are selected from: adenosine deaminase, asparagine synthetase, E.Coli trpB gene, Salmonella hisD gene, M2 mouse ribonucleotide reductase, human multidrug resistance gene, glutamine synthetase, xanthine guanine phosphoribosyl transferase, hygromycin B, neomycin gene, dihydrofolate reductase and neomycin.
6. A CHO cell-line as claimed in any of Claims 1-5 wherein one of the markers provides a basis for amplification.
7. A CHO cell-line as claimed in any of Claims 1-6 wherein the cell-line is dhfr⁻ phenotype and one of the markers is dhfr.

8. A CHO cell-line as claimed in Claim 7 wherein the second marker is neomycin.
9. A CHO cell-line as claimed in Claims 1 or 2 wherein the antibody is a chimaeric or a CDR-grafted antibody.
10. A CHO cell-line as claimed in either of Claims 1 or 2 capable of producing an antibody against a T-cell marker or a tumour cell marker.
11. A CHO cell-line as claimed in Claim 10 wherein the antibody is raised against an antigen selected from CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD11b, CD18, CD19, CD25, CD33, CD38, CDw52 and CD54.
12. A CHO cell-line as claimed in Claim 11 capable of producing an anti-CD_w52 antibody.
13. A human or altered antibody, except a CDR-grafted anti-CD4 antibody, having CHO glycosylation.
14. An antibody as claimed in Claim 13 wherein the altered antibody is a chimaeric or a CDR-grafted antibody.
15. An antibody as claimed in Claim 14 which is an anti-CD_w52 antibody.
16. A process for the preparation of an antibody as defined in any of Claims 13 to 15 comprising culturing a CHO cell engineered to express the antibody and recovering the antibody from the culture medium.
17. An antibody as defined in any of Claims 13 to 15 for use in therapy.

18. Use of an antibody as defined in any of Claims 13 to 15 in the manufacture of a medicament for immunosuppression.
19. Use of an antibody as claimed in Claim 18 for the treatment of T-cell mediated disorders, severe vasculitis, rheumatoid arthritis, systemic lupis, multiple sclerosis, graft vs. host disease, psoriarsis, juvenile onset diabetes, Sjorgren's disease, thyroid disease, myasthenia gravis, transplant rejection or asthma.
20. Use of an antibody as defined in any of Claims 13 to 15 in the manufacture of a medicament for the treatment of cancer.
21. Use of antibody as claimed in any of Claims 18 to 20 wherein the antibody is an anti-CD_w52 antibody.
22. Use of an antibody as defined in any of Claims 13 to 15 in the manufacture of a medicament for the treatment of infectious diseases.
23. A formulation containing a CHO-glycosylated antibody as defined in any of Claims 13 to 15 and a physiologically acceptable diluent or carrier.

FIGURE I/5

7

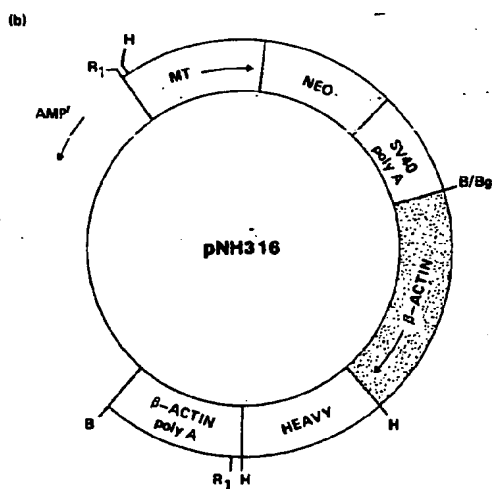
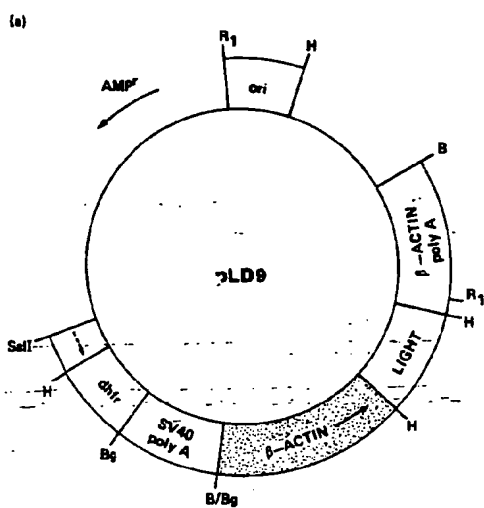


FIGURE II / 5

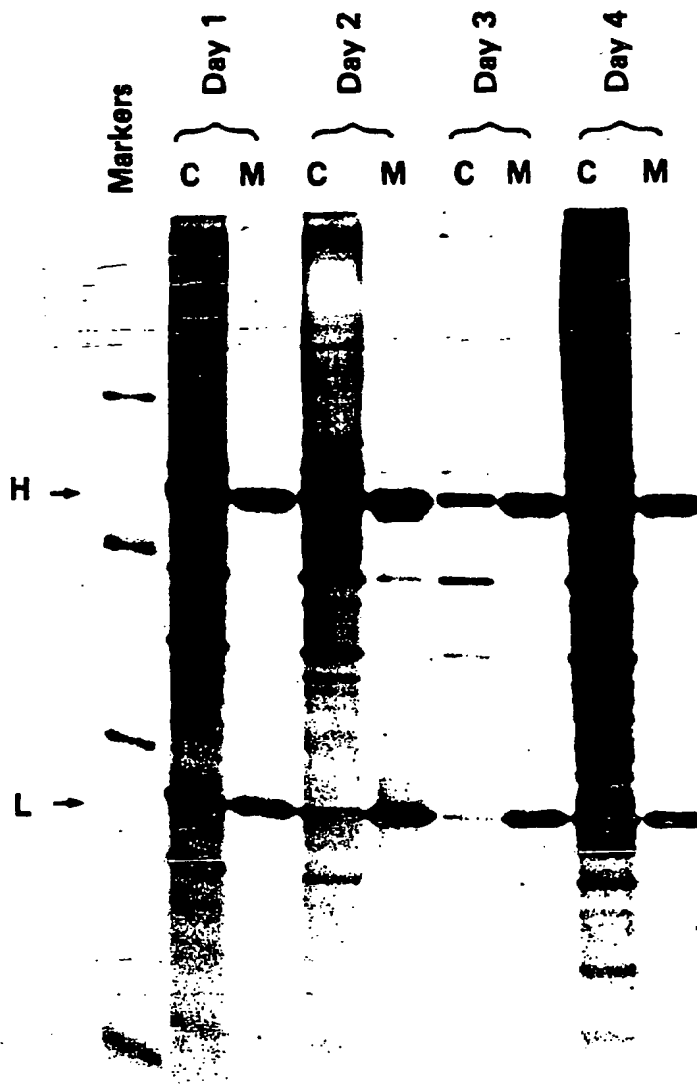
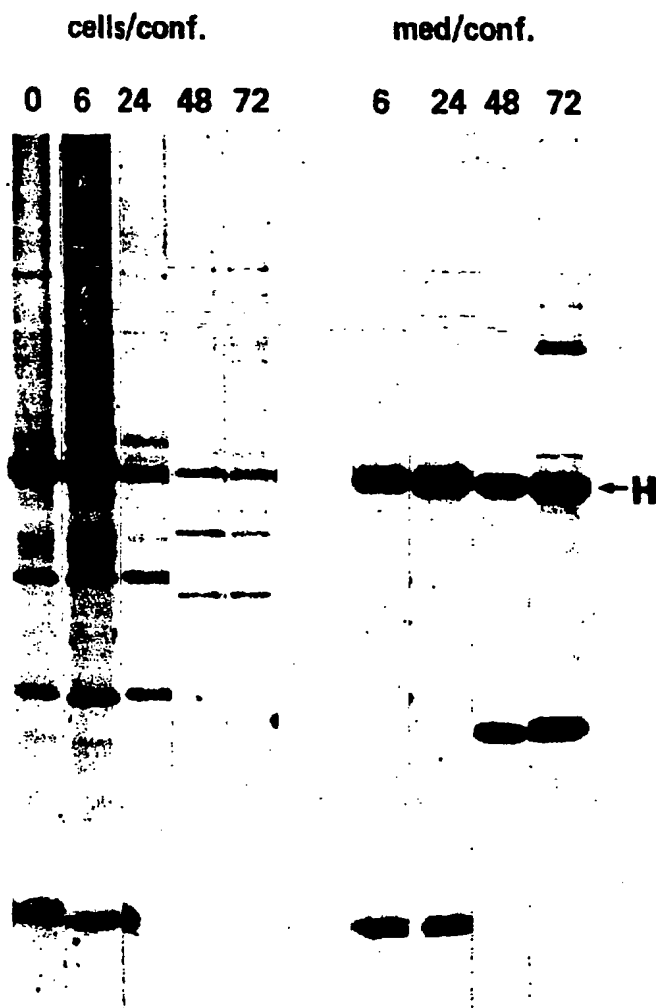


FIGURE III /5



THE PR-

4/5

FERMENTER ADAPTION OF CAMPATH 1H CELLS

LINE 44 (NAF170/181)

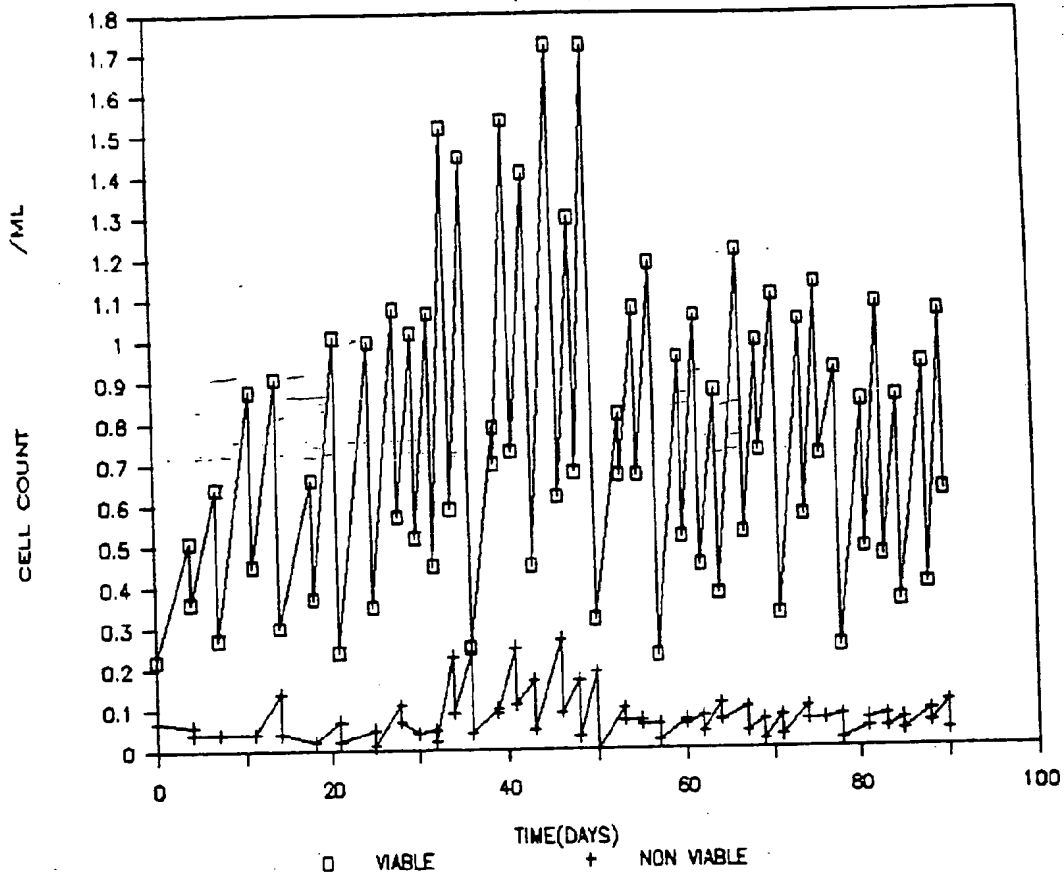


FIGURE 4

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FERMENTER ADAPTION OF CAMPATH 1H CELLS

LINE 44(NAF170/181)

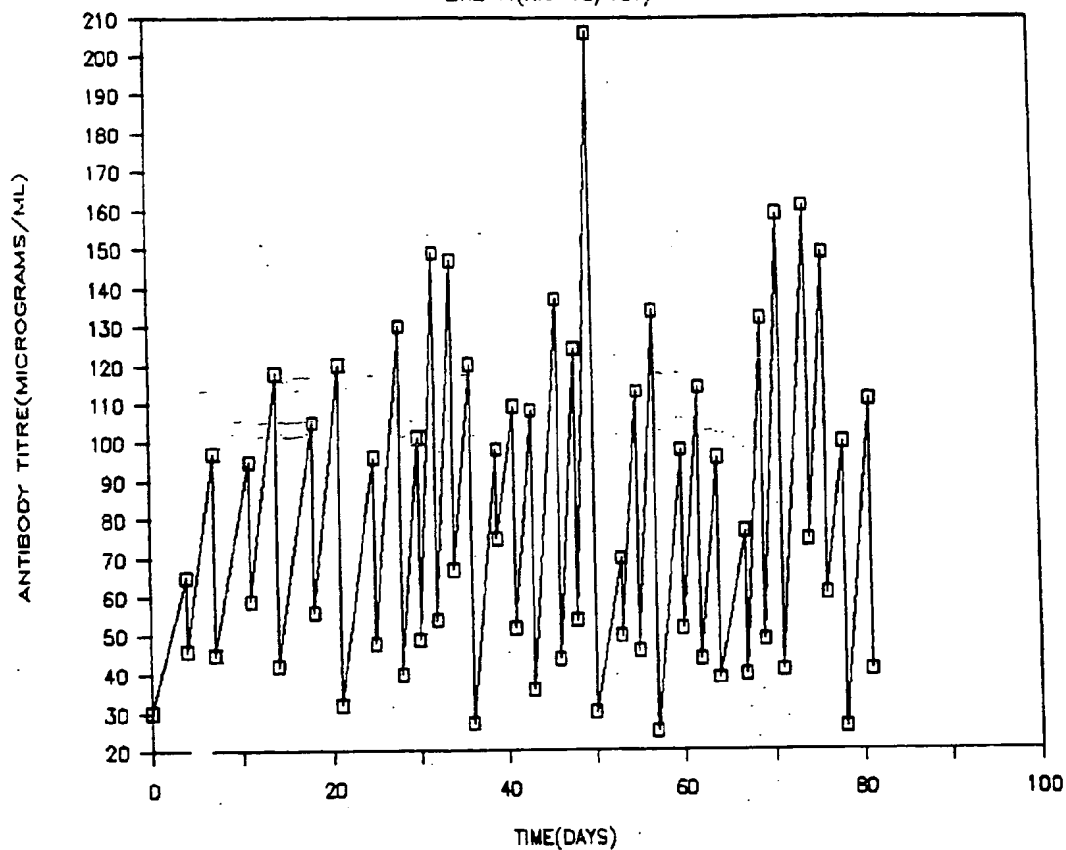


Figure 5